Claims 50-52 are pending in this application. Claims 58-61, 65-67 and 72, which are withdrawn from consideration as being drawn to non-elected subject matter, are cancelled herein. Applicant reserves the right to file divisional applications to the non-elected subject matter.

Claim 50 is amended to more particularly point out the claimed subject matter. No amendments have been made to obviate prior art and no new matter has been introduced.

An executed DECLARATION under 37 C.F.R. §1.132 of Fabijanski, is attached hereto, as is a marked up copy per 37 C.F.R. §1.121 of the amended claims. A Supplemental Information Disclosure Statement also is filed on the same day herewith under separate cover.

REQUIREMENT FOR RESTRICTION

A Requirement for Restriction was set forth in connection with the above-captioned application on October 3, 2002. In the Response to the Requirement for Restriction filed November 1, 2002, Applicant elected, with traverse, Group IV, Claims 50-52, drawn to a method of producing a transgenic plant by introducing a SATAC into a pl plant cell.

Traversal of the Restriction Requirement

The Examiner was not persuaded by Applicant's traversal of the above Requirement for Restriction and has made the Requirement for Restriction Final. Specifically:

(A) Responsive to Applicant's arguments that Groups IV and VIII are not independent or distinct because plant cells and protoplasts are related as genus/species, the Examiner alleges that protoplasts are not plant cells because, as "Applicant even points out,...a plant cell has a cell wall, which a protoplast does not." It is further alleged that a protoplast is "physically, biochemically, structurally" distinct from a plant cell. The Examiner has not responded to

Applicant's traversal as to the Restriction Requirement between Groups VIII and VI.

Applicant respectfully maintains that the claims of Groups IV and VIII are related as genus/species. In the response to the Restriction Requirement of October 3, 2002, Applicant did not distinguish a plant cell from a protoplast as being distinct. To the contrary, Applicant said that "A protoplast is a plant cell from which the cell wall is removed." (page 3, line 12 of the response filed November 1, 2002; emphasis added). Further, those of skill in the art at the time the instant application was filed and as of its earliest priority date considered protoplasts to be plant cells, and the Examiner has provided no evidence to the contrary. For example, several of the references discussed below in connection with the rejection on grounds of enablement describe the manipulation of protoplasts to introduce heterologous DNA into the protoplasts and/or generate transgenic plants therefrom as experimental work involving plant cells (*see, e.g.*, Schmidt *et al.* (1996) *Plant J. 9*:683-691; Houba-Hérin *et al.* (1994) *Plant J. 6*:55-66; Funatsuki *et al.* (1995) *Theor. Appl. Genet. 91*:707-712; and Zhang *et al.* (1988) *Plant Cell Reports 7*:379-384, which describe transformed protoplasts as transformed cells; Datta *et al.* (1990) *Bio/Technology 8*:736-740, which describes protoplast culture as "cell culture"; Negrutiu *et al.*, *Int. J. Dev. Biol.*, 36:73-84 (1992), which describes protoplasts as "wall-less" cells that can divide mitotically, proliferate clonally and produce a complete plant; and Rogers *et al.*, "Methods for Plant Molecular Biology," Academic Press (1988) VIII(26):423-436, which describes protoplasts as plant cells from which transformed plants are produced). In all of the aforementioned references, protoplasts are subjected standard techniques for manipulating cells and they are referred to as cells or plant cells.

Further, contrary to the Examiner's assertions, a protoplast does not exhibit biochemical, physical or structurally different properties from a plant cell. For example, Negrutiu *et al.* point out how plant protoplasts can be manipulated

to generate mutant varieties of seeds (which produce plants) by introducing foreign DNA into their host genomes, culturing them, performing gene expression studies, and subjecting them to molecular cytogenetics and flow cytometry (*see* p. 73). In addition, for example, page 51, lines 3-9 of the specification describes and incorporates by reference (*Ag Biotechnology News* 7:3 and 17 (September/October 1990) how electroporation is often used for transformation of plants. The cited passage describes how electroporated transformed plant protoplasts reform the cell wall, divide, and form plant callus. A protoplast is a plant cell because it can regenerate into a plant. Accordingly, Applicant respectfully maintains that Group VIII, which is directed to a method for producing a transgenic plant by introducing a SATAC into a plant cell; and producing a transgenic plant therefrom and Group IV, which is directed to a method for producing a transgenic plant by introducing a SATAC into a protoplast; and producing a transgenic plant therefrom, are not independent or distinct because a protoplast is a plant cell and thus, Group IV is a species of the genus encompassed by Group VIII.

Applicant further maintains that as to the claims of Group VIII and Group VI, the restriction requirement is improper. Group VIII is directed to a method for producing a transgenic plant by introducing a SATAC into a plant cell; and producing a transgenic plant therefrom. Group VI is directed to a method for producing a transgenic plant by introducing SATAC into a plant cell. Group VI claims include the steps by which a SATAC can be made. Therefore, Group VI is directed to species of Group VIII in which the SATAC that is introduced in the plant cell is made by the steps specified in the claim. Thus, Group VIII and Group VI are related as a genus/species, and are not properly restrictable.

(B) Responsive to Applicant's arguments that the sequences of Group II are improperly restricted because they do not encode different proteins, the Examiner alleges that the sequences "differ structurally" and would therefore each require a separate search, regardless of whether they encode a protein.

Applicant respectfully submits that according to MPEP §803.04, claims drawn to nucleotide sequences encoding different proteins are deemed properly restrictable. Accordingly, Applicant maintains that each of SEQ ID Nos. 18-24 of the claims of Group II, which do not encode different proteins but are portions of rRNA-encoding DNA, are not independent or distinct.

It is noted that if the Patent Office maintains this Restriction Requirement, the Office is now precluded from holding obviousness-type double patenting as between the instant application and any divisional application(s) claiming any subject matter of Claims 58-61, 65-67 and 72. These claims are cancelled herein in reliance upon the Examiner's holding that the Requirement is made final.

THE REJECTION OF CLAIMS 50-52 UNDER 35 U.S.C. § 112, Second Paragraph

Claims 50-52 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter. Specifically, the Office Action alleges that the "protoplast cell" lacks antecedent basis. Amendment of Claim 50 to recite "protoplast" instead of "protoplast cell" obviates this rejection.

The Office Action further alleges that Claim 50 is an incomplete method claim because essential steps of the method are not recited. This rejection is respectfully traversed.

RELEVANT LAW

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). When one skilled in the art would understand all of the language in the claims when read in light of the specification, a claim is not indefinite.

There are no requirements for terms to be defined in the claims when one of skill in the art can readily determine the meaning of the term based on the description and definitions provided in the specification. In this respect, an Applicant is entitled to be its own lexicographer (see, *e.g.*, MPEP 2111.01 "Applicant may be his or her own lexicographer as long as the meaning assigned to the term is not repugnant to the term's well known usage and utilize terms within the claims that are clear from a reading of the specification. *In re Hill*, 73 USPQ 482 (CCPA 1947)."). When Applicant has provided definitions in the specification, the claims are interpreted in light of such definition.

35 U.S.C. § 112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Col.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

The amount of detail required to be included in the claims depends on the particular subject matter and the prior art and is not to be viewed in the abstract, but in conjunction with whether the specification is in compliance with the first paragraph of 35 U.S.C. § 112. If the claims, read in light of the specification, reasonably apprise those skilled in the art of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more:

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (*Bendix Corp. v United States*, 600 F.2d 1364, 1369, 220 Ct. Cl. 507, 514, 204 USPQ 617, 621 (1979); See, also, *Carl Zeiss Stiftung v. Renishaw plc*, 20 USPQ2d 1094, 1101).

ANALYSIS

As noted above, definiteness of claim language must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure,

(2) the teachings of prior art and (3) the interpretation claims would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. The instant claims are definite because the steps of (i) introducing a satellite artificial chromosome into a plant protoplast and (ii) growing the protoplast under conditions to produce a transgenic plant adequately define the metes and bounds of the claimed subject matter in light of the disclosure in the specification and what is known to those of skill in the art.

Methods of introducing satellite artificial chromosomes into plant cells are provided in great detail throughout the specification. As the specification describes and incorporates by reference, introduction of heterologous DNA into plant cells can be accomplished using a variety of standard techniques, such as is described in the specification (see, *e.g.*, page 54, line 1, through page 55, line 3; page 48, lines 11-29; and page 50, line 17, through page 51, line 14) and in the incorporated references (see, *e.g.*, Uchimiya *et al.* (1989) *J. of Biotech. 12*: 1-20; Weissbach *et al.* (1988) Methods for Plant Molecular Biology, Academic Press, N.Y., Section VIII, pp. 421-463; Grierson *et al.* (1988) Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9; and U.S. Patent Nos. 5,491,075; 5,482,928; 5,424,409; 5,436,392; and 5,489,520, of record in the file history of the instant application). Further, the preparation of satellite artificial chromosomes is described and illustrated in great detail throughout the specification and drawings (see, *e.g.*, page 33, line 19, through page 36, line 23; the EXAMPLES, particularly EXAMPLES 4-7; and Figures 2 and 3).

Methods of producing a transgenic plant from a plant cell such as a plant protoplast were well known to those of skill in the art at the time of filing of the above-captioned application and as of its earliest priority date. As the specification recites, the method used for producing a transgenic plant is primarily a function of the species of plant (exemplary species of plant are

provided in the specification, see, *e.g.*, page 54, lines 12-16) and protocols used to introduce DNA into plant cells and produce a transgenic plant are readily adapted by one of skill in the art. Further, the specification incorporates by reference, methods of producing transgenic plants (see, *e.g.*, U.S. Patent Nos. 5,489,520 and 5,482,928).

Furthermore, as discussed below in addressing the rejection for lack of enablement, methods for introducing heterologous DNA into plant cells such as plant protoplasts and conditions for generating transgenic plants from the transformed protoplasts were well known in the art at the time of filing of the above-captioned application and as of its earliest priority date. As of the earliest priority date of the above-captioned application, those of skill in the art knew that plant cells could readily be transformed and induced to generate a variety of plants, and cell growth conditions for plant generation were well known (*see, e.g.*, Negrutiu *et al.*, *Int. J. Dev. Biol.*, 36:73-84 (1992); Rogers *et al.*, "Methods for Plant Molecular Biology," Academic Press (1988) VIII(26):423-436; Zupan *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:2392-2397 (1992)). Procedures for introducing heterologous DNA into tobacco protoplasts, using such methods as electroporation or PEG-mediated transformation, and the subsequent generation of transgenic tobacco plants from the transformed tobacco protoplasts were available (see, *e.g.*, Schmidt *et al.* (1996) *Plant J. 9*:683-691; Houba-Hérin *et al.* (1994) *Plant J. 6*:55-66; EP0392225). Similarly, methods for transformation of a variety of different plant cell types, including rice, maize, carrot, sunflower, tomato, cotton, corn and barley protoplasts, and the generation of transgenic plants from such protoplasts also were available (Zhang *et al.* (1988) *Plant Cell Reports 7*:379-384; Shimamoto *et al.* (1989) *Nature 338*:274-276; Datta *et al.* (1990) *Bio/Technology 8*:736-740; Funatsuki *et al.* (1995) *Theor. Appl. Genet. 91*:707-712; EP0292435; EP0392225; and WO 93/07278).

Therefore, it is respectfully submitted that one of skill in the art, given the steps of (i) introducing a satellite artificial chromosome into a plant protoplast and (ii) growing the protoplast under conditions to produce a transgenic plant, would recognize (i) the scope/boundaries of the claimed subject matter; and (ii) the standard techniques by which the introduction of astellite artificial chromosomes into a plant cell and the production of a transgenic plant therefrom may be effected. No further elaboration is necessary to render definite each of the steps of the claimed method.

It is respectfully submitted that in this instance, the claims reasonably apprise the skilled artisan of the scope and utilization of the claimed method. The steps of (i) introducing a satellite artificial chromosomes into a plant protoplast and (ii) growing the protoplast under conditions to produce a transgenic plant involve well-known methods and/or methods that are clearly detailed in the specification. Thus, they need not be further elucidated for practical utilization of the claimed subject matter. Therefore, it is respectfully submitted that Claim 50 and claims dependent thereon (Claims 51 and 52) are not indefinite.

THE REJECTION OF CLAIMS 50-52 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 50-52 are rejected under 35 U.S.C. § 112, first paragraph because it is alleged that the specification does not reasonably provide enablement for <u>any</u> satellite artificial chromosomes in <u>any</u> plant protoplast or <u>any</u> transgenic plant. In particular, the Office Action alleges (1) that the differences between plants and animals, particularly their respective satellite DNA, telomeres, centromeres and heterochromatin, make it unpredictable that a plant would have centromeres that are structurally and biochemically the same as those of animals; and (2) that the alleged complexity of satellite artificial chromosomes,

especially their large sizes, when combined with other components of heterologous expression constructs such as different promoters, enhancers, codon optimization, termination regions and other regulatory regions, would lead one of skill in the art to expect that a satellite artificial chromosome constructed for mammalian cells would differ from a satellite artificial chromosome that is constructed for a plant cell. To support this allegation, the Office Action cites numerous (post filing date) references that allegedly demonstrate the differences between plant and animal DNA and chromosomal components and the difficulties in transferring large pieces of DNA between cells (see, *e.g.*, Ferl *et al.* in Buchanan *et al.* "*Biochemistry and Molecular Biology of Plants*" (2000) American Society of Plant Physiologists, Rockville MD, pg. 324; Lehninger "*Biochemistry*", 2nd Edition (1976) Worth Publishers, New York, pg. 864; Willard *Science 290:* 1308-1309 (2000); Copenhaver *et al. Science 286:* 2468-2474 (1999); Shen *et al. Current Biology 10:* 31-34 (2000); Telenius *et al. Chromosome Res. 7*: 3-7 (1999); Avramova *et al. Plant Physiology 129:* 40-49 (2002); Brown *Trends in Biotech. 18:* 403 (2000); Perez *et al. Trends in Biotech 18*: 402-403 (2000); and Hadlaczky *Curr. Opin. Mol. Ther. 3:* 125-132 (2001)).

The Office Action further alleges that while the specification provides methods for the preparation and transfer of an animal satellite artificial chromosome into a mammalian cell (human, mouse and hamster cells), there is no evidence that these methods produce a satellite artificial chromosome from any source (*i.e.*, animals or plants) that is operable in any cell type (*i.e.*, an animal or plant cell). It is alleged that while one of skill in the art can readily make changes to Applicant's animal satellite artificial chromosomes to generate a non-animal satellite artificial chromosome, there is no guidance as to what these changes should be, leading one of skill in the art to make random changes fraught with trial and error, requiring undue experimentation. To support this allegation, the Office Action cites Willard (*Science 290:* 1308-1309 (2000)),

which allegedly demonstrates the state of the art of artificial chromosome technology.

The Office Action concludes that the specification is not enabling for a satellite artificial chromosome that is universally adapted to be operable in all cells. The Office Action further concludes that, due to the unpredictability that the satellite artificial chromosome provided by Applicant would be operable in all cell types, Applicant has only enabled a satellite artificial chromosome for use in a mammalian cell and not in a plant protoplast or transgenic plant.

This rejection is respectfully traversed. Although not needed, a DECLARATION under 37 C.F.R. §1.132 of Fabijanski demonstrating the ability to introduce satellite artificial chromosomes into plant cells and retain the satellite artificial chromosomes in plant cells is provided.

First it is noted that the instant application is a divisional of copending U.S. application Serial No. 08/835,682, filed April 10, 1997, is also a continuation-in-part of U.S. application Serial No. 08/695,191, filed August 7, 1996, now U.S. Patent No. 6,025,155, is also continuation-in-part of U.S. application Serial No. 08/682,080, filed July 15, 1996, now U.S. Patent No. 6,077,697, and is also a continuation-in-part of copending U.S. application Serial No. 08/629,822, filed April 10, 1996. The presently pending claims have an effective filing date of April 10, 1996.

Reliance upon post-filing date references to establish a lack of enablement is improper. Furthermore, reliance upon references such as Willard and references that describe other artificial chromosomes is inapt, since the technology therein is unrelated to the technology upon which the instant methods are based, and does not represent the state of the art of satellite artificial chromosome technology. It is the instant Applicant who first invented the satellite artificial chromosomes and they can be produced by a method, whose steps are outlined in detail in the specification, in any eukaryotic cell that contains chromosomes with amplifiable DNA; one does not have to know what

components are required or what telomeres are needed or other parameters. Cells, when treated as described in the instant application, generate chromosomal structures through an amplification event that result in chromosomal structures that produce satellite artificial chromosomes. Knowledge of the mechanism is not needed. Furthermore, the amplification event that leads to generation of satellite artificial chromosomes does occur in plants (see, *e.g.*, U.S. Patent Nos. 6,355,860 and 6,100,092, which demonstrate such event).

The instant Applicant is the first to identify such events and their use to generate satellite artificial chromosomes. As such, this is a pioneering invention entitled to broad scope.

Relevant law

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples **or** by broad terminology." In re Marzocci et al., 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an Applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is

no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims that are commensurate in scope not only with what Applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the Applicant has disclosed.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

ANALYSIS

As demonstrated below, it would not require undue experimentation to (i) generate animal or plant satellite artificial chromosomes; (ii) introduce animal or plant satellite artificial chromosomes into plant cells; and (iii) grow the plant cells under conditions to produce transgenic plants, that are within the scope of the claims, in view of the knowledge and level of skill in the art and the teachings and disclosure in the specification regarding methods for generating satellite artificial chromosomes for use in different species; methods for delivery of

artificial chromosomes to selected cells, including, for example, the cells of a host plant, animal or insect; methods for cloning centromeres from different species for use in the preparation of species-specific satellite artificial chromosomes; methods for producing cells of different species that contain heterologous nucleic acids such as satellite artificial chromosomes; and methods for generating transgenic plants from plant cells into which satellite artificial chromosomes have been introduced.

As demonstrated below, the teachings of the specification, when taken in conjunction with what is known to one of skill in the art, are such that it would not require undue experimentation to perform the steps of the methods claimed herein, including the preparation and isolation of animal or plant satellite artificial chromosomes, their introduction into a plant cell such as a plant protoplast of any species and the growth of the plant cell under conditions to produce transgenic plants containing a satellite artificial chromosome.

The level of knowledge and skill in the construction, introduction into cells, including plant cells, and stable expression of large sizes of DNA, as well as the production of transgenic plants, was so high as of the effective filing date that it would not have required extensive experimentation by one of skill in the art to produce plant satellite artificial chromosomes by the methods in the working examples and the publications incorporated herein by reference, nor would it have required extensive experimentation to transfer the resulting plant satellite artificial chromosome into a plant cell in order to produce a transgenic plant. Furthermore, the specification describes methods for generation and identification of satellite artificial chromosomes, exemplifies such methods with respect to mammalian chromosomes, and provides a broad disclosure that can be practiced with any eukaryotic cell. It is only necessary to introduce a piece of DNA and a selectable marker into a cell, grow the cell under selective conditions, look for cells that contain satellite artificial chromosomes, select such cells, and isolate a satellite artificial chromosome therefrom. There is no

evidence of record that suggests that such events are unique to animal cells, nor is there any reason to believe such. On the contrary, the specification teaches that the events and the methods based thereon are applicable to cells from any eukaryotic species. As noted above, the generation of a satellite artificial chromosome requires an amplification event and there is no reason provided by the Examiner nor of record that suggests that plant chromosomes do not undergo amplification. In fact, plants do have amplifiable regions (see, *e.g.*, U.S. Patent Nos. 6,355,860 and 6,100,092).

Scope of the claims

The claims are directed to methods for producing a transgenic plant by introducing a satellite artificial chromosome into the plant protoplast and growing the plant protoplast under conditions to produce a transgenic plant. The dependent claims specify that the satellite artificial chromosomes comprise heterologous DNA that encodes a gene product; and particular methods for the introduction of the satellite artificial chromosomes into plant protoplasts.

Teachings of the specification

The specification describes the production, characterization and isolation of satellite artificial chromosomes and their transfection into cells. The teachings of the specification describe how to: (i) prepare satellite artificial chromosomes with heterologous nucleic acid incorporated therein, (ii) transfer the satellite artificial chromosome containing the heterologous nucleic acid into a cell; and (iii) determine the expression of a gene product(s) encoded by the satellite artificial chromosome.

Each of these steps are described in detail in the specification. In addition, the specification provides numerous working examples of the procedures and results involved in the claimed methods. For example, the specification discloses methods for generating artificial chromosomes, such as satellite artificial chromosomes; methods for generating species-specific satellite artificial chromosomes; methods for cloning centromeres from particular

sources; methods for isolation and large-scale production of artificial chromosomes; methods for delivery of artificial chromosomes to selected cells, including, for example, the cells of a host plant, animal or insect; and methods for the expression of products encoded by the nucleic acids of the artificial chromosomes in cells, including the cells of a host animal, plant, or insect. Further, the specification provides cell lines and chromosomes produced by the methods described, which can be used as vehicles for the expression of heterologous nucleic acids in cells *in vitro* and *in vivo*.

The specification provides methods of generating satellite artificial chromosomes, and characterizes in exquisite detail the artificial chromosomes generated by such methods. To illustrate the methods and products thereof, the specification describes the exact procedures used to generate multiple specific cell lines containing satellite artificial chromosomes (see, *e.g.*, Examples 2-7, beginning at page 75, line 8), and Applicant provides to the public no less than six of the described cell lines that have been deposited at an authorized depository (*i.e.*, the European Collection of Animal Cell Culture) (see, *e.g.*, page 74, line 23, through page 75, line 7).

The specification teaches the development of a satellite artificial chromosome containing sequences that can be of animal or plant origin and contain centromere-related sequences. For example, the specification provides methods for generating species-specific satellite artificial chromosomes by adding a centromere from other species, including plants (see, *e.g.*, at page 12, lines 9-22). In the specification, *e.g.*, at page 12, lines 5-22, a method for cloning a centromere in a selected species (*e.g.*, plants) is described. These methods for cloning centromeres from a selected animal or plant include: (i) preparing a library of DNA fragments that contains the genome of the plant or animal; (ii) introducing each of the fragments into a mammalian satellite artificial chromosome that contains a selectable marker and a centromere from a species different from the selected plant or animal; (iii) introducing each of the satellite

artificial chromosomes into a cell, which is grown under selective conditions; and (iv) selecting cells containing satellite artificial chromosomes. Satellite artificial chromosomes that are identified by the methods provided herein should contain a centromere encoded by the DNA from the library, be it plant DNA or mammalian DNA, and should contain the necessary elements for stable replication in the selected species. Thus, the specification provides methods where a satellite artificial chromosome is developed in one source cell, modified to contain sequences specific to the centromere region of a target cell and then transferred to the target cell type.

Methods for the modification of satellite artificial chromosomes are described in extensive detail in the specification (*e.g.*, at page 39, line 25, through page 40, line 21; and page 150 through page 157). The cited passage(s) describe the use of homologous recombination to insert new DNA *fragments into a satellite artificial chromosome. Accordingly, methods to* generate and modify a satellite artificial chromosome to contain a plant centromere DNA sequences have been provided.

The specification further teaches methods for producing satellite artificial chromosomes that contain heterologous DNA and the expression of the heterologous DNA contained therein in cells (see, *e.g.*, page 39, line 25, through page 41, line 3; page 61, line 26, through page 62, line 7; page 150, line 1, through page 165, line 12 and Example 12 beginning on page 140). Procedures for the isolation of artificial chromosomes (see, *e.g.*, page 41, line 4, through page 42, line 3; page 32, lines 13-24; page 80, lines 19-27; and Example 10, beginning on page 124) and for the transfer of the artificial chromosomes into cells (see, *e.g.*, page 10, line 30, through page 11, line 6; page 48, line 11, through page 51, line 26; page 52, line 11, through page 55, line 3; page 70, line 14, through page 72, line 27; and Example 13 beginning on page 165) are also described in detail in the specification.

At page 54, line 1, through page 55, line 3, the specification describes how to introduce satellite artificial chromosomes into plant cells by methods, such as direct transfer of DNA by processes, PEG-induced DNA uptake, protoplast fusion, microinjection, electroporation, and microprojectile bombardment, such as particle gun bombardment. Further, the accompanying DECLARATION under 37 C.F.R. § 1.132 of Steven F. Fabijanski demonstrates that by following the steps of the instant methods, one of skill in the art can introduce animal satellite artificial chromosomes into plant protoplasts using methods known to those of skill in the art at the time of filing of the above-captioned application and as of its earliest filing date, including microcell-mediated fusion and lipid-mediated transfection.

Methods of producing a transgenic plant from a plant cell such as a plant protoplast were well known to those of skill in the art at the time of filing of the above-captioned application and as of its earliest priority date. As the specification recites, the method used for producing a transgenic plant is primarily a function of the species of plant (exemplary species of plant are provided in the specification, see, *e.g.*, page 54, lines 12-16) and protocols used to introduce DNA into plant cells and produce a transgenic plant are readily adapted by one of skill in the art. Further, the specification incorporates by reference, methods of producing transgenic plants (see, *e.g.*, U.S. Patent Nos. 5,489,520 and 5,482,928).

The satellite artificial chromosomes delivered to and/or contained within the cells were extensively characterized using methods including Southern hybridization, long-range mapping of restriction endonuclease sites, indirect immunofluorescence with anti-centromere antibodies, *in situ* hybridization, analysis of G-band patterns, and chromosome painting. Such extensive analysis provides definition of the satellite artificial chromosomes at the level of the basic structural and functional elements of these chromosomes, including repeated

units of satellite and foreign DNA. Many of these features are depicted schematically in Figures 1-3 of the application.

By following the methods set forth in the specification, the specification teaches one of skill in the art how to generate satellite artificial chromosomes from various species (*e.g.*, plants and animals); readily identify the resulting satellite artificial chromosomes based on the detailed characterization provided in the specification; incorporate foreign nucleic acid (*e.g.*, heterologous DNA encoding a product into an artificial chromosome); isolate and transfer artificial chromosomes into cells from various species (*e.g.*, plants and animals) and grow plant cells under conditions that produce a transgenic plant. Thus, the teachings of the specification provide how to make and use the satellite artificial chromosomes and to combine these artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the introduction and long-term expression of satellite artificial chromosomes encoding products in cells of host animals, plants and insects and the generation of transgenic animals or plants from these cells.

Level of Skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art.

Knowledge of those of skill in the art

At the time of filing of the application and before, one of skill in the art knew the biochemical and structural properties of the various components of chromosomes from a wide variety of species (*e.g.*, plants, animals, bacteria and yeast). Further, there was a broad body of knowledge, set forth below and incorporated by reference into the instant specification, that was directed to the structure and function of native and artificial chromosomes from various sources

(*e.g.*, animal, bacteria, yeast and plant). Also known to those of skill in the art were methods for the manipulation of DNA, recombinant DNA techniques, techniques for the transfer of DNA into cells, including plant cells, and the production of transgenic plants from plant cells such as protoplasts containing heterologous DNA.

For example, sequence information for plant centromeres, telomeres and autonomously replicating sequences (ARS) was available (see, *e.g.*, Jiang *et al. Proc Natl Acad Sci USA, 93*:14210-14213 (1996); Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); Zakian *Science 270(5242):* 1601-1607 (1995); Berlani *et al. Plant Mol. Biol. 11:* 161-162 (1988); Berlani *et al. Plant Mol. Biol. 11:* 173-182 (1988); and Eckdahl *et al. Plant Mol. Biol. 12:* 507-516 (1989)). Methods for the isolation of plant centromere DNA sequences were also available (see, *e.g.*, Jiang *et al. Proc Natl Acad Sci USA, 93*:14210-14213 (1996); Kaszas *et al. EMBO J. 15:* 5246-5255 (1996); Frary *et al. Mol. Genet. 250:* 295-340 (1996)). In these methods, the centromeres are localized either by genetically analyzing the assortment of chromosome fragments and rearrangements or by cytological analysis. In the specification, *e.g.*, at page 12, lines 5-22, a method for cloning a centromere in a selected species (*e.g.*, plants) is described. In addition, methods for the production of repeated tandem arrays of DNA, such as telomeric DNA, are also provided in the specification (see, *e.g.*, page 14, line 4, through page 15, line 8; and page 64, line 19, through page 68, line 27).

Procedures relating to DNA manipulation are found throughout the application as seen, *e.g.*, at page 73, line 23, through page 74, line 21, and incorporated by reference (see, *e.g.*, Sambrook *et al.* (1989) *Molecular cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press,* Cold Spring Harbor, NY, Wong *et al.* (1988) *Nucl. Acids Res. 16*:11645-11661, Fatyol *et al.* (1994)

Nucl. Acids Res. 22:3728-3736, Morgenstern *et al.* (1990) *Nucl. Acids Res. 18*:3587-3596, Tonghua *et al.* (1995) *Chin. Med. J. (Beijing, Engl. Ed.) 108*:653-659, Couto *et al.* (1994) *Infect. Immun. 62*:2375-2378, Dunckley *et al.* (1992) *FEBS Lett. 296*:128-34, French *et al.* (1995) *Anal. Biochem. 228*:354-355, Liu *et al.* (1995) *Blood 85*:1095-1103, International PCT application Nos. WO 95/20044, WO 95/00178, and WO 94/19456). These methods are not specific for the preparation of vectors and plasmids for use in a mammalian systems. Use of these methods or alteration of the methods described in the instant application to prepare vectors and plasmids for use different systems, such as, for example, a plant system, are known to those of skill in the art.

Procedures for the introduction of satellite artificial chromosomes into host plant and animal cells are referred to in many instances throughout the application. For example, the application references numerous procedures for the introduction of satellite artificial chromosomes into cells (see, *e.g.*, at page 23, line 18, through page 24, line 11; at page 48, line 11, through page 51, line 26; and at page 54, line 1, through page 55, line 3), including the direct uptake of DNA using calcium phosphate (*e.g.*, Wigler *et al.* (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*:1373-1376), electroporation, lipid mediated transfer, *e.g.*, lipofection and liposomes, polyethylene glycol-mediated DNA uptake (*e.g.*, Strauss (1996) *Meth. Mol. Biol. 54*:307-327; U.S. Patent Nos. 4,684,611; 5,491,075; 5,482,928; and 5,424,409), microcell fusion (*e.g.*, Lambert (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*:5907-5911; U.S. Patent No. 5,396,767; Sawford *et al.* (1987) *Somatic Cell Mol. Genet. 13*:279-284; Dhar *et al.* (1984) *Somatic Cell Mol. Genet. 10*:547-559; and McNeill-Killary *et al.* (1995) *Meth. Enzymol. 254*:133-152), lipid-mediated carrier systems (*e.g.*, Teifel *et al.* (1995) *Biotechniques 19*:79-80; Albrecht *et al.* (1996) *Ann. Hematol. 72*:73-79; Holmen *et al.* (1995) *In Vitro Cell Dev. Biol. Anim. 31*:347-351; Remy *et al.* (1994) *Bio Conjug. Chem. 53*:647-654; Le Bolch *et al.* (1995) *Tetrahedron Lett.*

36:6681-6684; Loeffler *et al.* (1993) *Meth. Enzymol. 217*:599-618), microprojectile bombardment (*e.g.*, Uchimiya *et al.* (1989) *J. of Biotech. 12*: 1-20; U.S. Patent Nos. 5,436,392; 5,489,520; and 5,470,708), microinjection in cells and embryos and protoplast regeneration for plants (*e.g.*, Weissbach *et al.* (1988) *Methods for Plant Molecular Biology*, Academic Press, N.Y., Section VIII, pp. 421-463; and Grierson *et al.* (1988) *Plant Molecular Biology, 2d Ed.,* Blackie, London, Ch. 7-9). Other methods described in the application for introducing satellite artificial chromosomes into cells include nuclear microinjection and bacterial protoplast fusion with intact cells. Polycations, such as polybrene and polyornithine, also can be used.

At page 49, line 14, to page 51, line 26, the specification describes methods for introducing satellite artificial chromosomes into particular cell types using standard techniques appropriate for each type of cells. For example, for plant cells, methods for direct gene transfer into plant cells include polyethylene glycol (PEG)-mediated DNA uptake, electroporation-mediated DNA uptake and microinjection. In addition, plants may be transformed using ultrasound treatment (see, *e.g.*, International PCT application publication No. WO 91/00358).

For example, at page 50, line 24, through page 51, line 14, the specification describes the use of electroporation for transformation of plants (see, *e.g.*, *Ag Biotechnology News 7*:3 and 17 (September/October 1990)). In this technique, plant protoplasts are electroporated in the presence of the DNA of interest that also includes a phenotypic marker. Microinjection of DNA into plant cells, including cultured cells and cells in intact plant organs and embryoids in tissue culture and microprojectile bombardment (acceleration of small high density particles, which contain the DNA, to high velocity with a particle gun apparatus, which forces the particles to penetrate plant cell walls and membranes; see, *e.g.*, U.S. Patent Nos. 4,955,378, 4,923,814, 4,476,004,

4,906,576 and 4,441,972) have also been used. Direct transfer of DNA into cells also includes virion-mediated gene transfer methods.

Procedures for analysis of transformed cells to evaluate effectiveness of the introduction of heterologous nucleic acids are also described in the application. For example, at page 32, lines 9-11, page 38, lines 3-7, and page 70, lines 9-12, the use of *in situ* hybridization to detect the presence of specific chromosomes and/or specific DNA contained within specific chromosomes in cells is described. At page 82, lines 2-4, the use of PCR amplification and Southern blot techniques in confirming transfer of a satellite artificial chromosome into a host cell is described. At page 72, line 28, to page 73, line 22, the application references procedures for the detection and characterization of chromosomes (see, *e.g.*, Wang and Fedoroff (1972) *Nature 235*:52-54, Sumner (1972) *Exp. Cell Res. 75*:304-306, Perry and Wolff (1974) *Nature 251*:156-158, Hadlaczky *et al.*, (1986) *Exp. Cell Res. 167*:1-15, Hadlaczky *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*:8106-8110, U.S. application Serial No. 08/375,271, Sumner (1991) *Chromosoma 100*:410-418).

At page 89, lines 1-10, the application references procedures, such as Northern analysis and assays for foreign gene products, for assessing the specificity and level of expression in cells of a gene product. In Example 6, at page 103, the application references procedures, such as absorption spectrometry and phosphocellulose paper binding assays, for assessing the level of expression of β-galactosidase and hygromycin transferase gene products encoded by a satellite artificial chromosome.

Methods for introducing heterologous DNA into plant cells and generating transgenic plants from the transformed cells were well known in the art at the time of filing and before. For example, as of the earliest priority of the date of the above-captioned application, it was recognized that a variety of plant cells, including plant protoplasts, were versatile tools for introducing heterologous DNA, monitoring gene expression in the transformed cells, conducting

developmental studies and generating transgenic plants. As of the earliest priority date of the above-captioned application, those of skill knew that plant cells could readily be transformed and induced to generate a variety of plants (*see, e.g.*, Negrutiu *et al.*, *Int. J. Dev. Biol.*, 36:73-84 (1992); Rogers *et al.*, "Methods for Plant Molecular Biology," Academic Press (1988) VIII(26):423-436; Zupan *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:2392-2397 (1992)).

Procedures for introducing heterologous DNA into tobacco protoplasts, using such methods as electroporation or PEG-mediated transformation, and the subsequent generation of transgenic tobacco plants from the transformed tobacco protoplasts were available (see, *e.g.*, ten Lohuis *et al.*, *Plant J.*, 8(6):919-932 (1995); Schmidt *et al.* (1996) *Plant J. 9*:683-691; Houba-Hérin *et al.* (1994) *Plant J. 6*:55-66; EP0392225; Lepetit *et al.*, *Mol. Gen. Genet.*, 231:276-285 (1992)). Similarly, methods for transformation of a variety of different plant cell types, including petunia, rice, maize, carrot, sunflower, tomato, cotton, corn and barley protoplasts, and the generation of transgenic plants from such transformed cells also were available (ten Lohuis *et al.*, *Plant J.*, 8(6):919-932 (1995); Zhang *et al.* (1988) *Plant Cell Reports 7*:379-384; Shimamoto *et al.* (1989) *Nature 338*:274-276; Shimamoto *et al.* (1993) *Mol. Gen. Genet.*, 239:354-360; Datta *et al.* (1990) *Bio/Technology 8*:736-740; Funatsuki *et al.* (1995) *Theor. Appl. Genet. 91*:707-712; EP0292435; EP0392225; and WO 93/07278). As the specification states, the method used for producing a transgenic plant is primarily a function of the species of plant. At the time of filing, numerous protocols were available for producing transgenic plants. The protocols used to introduce DNA into plant cells and produce a transgenic plant are readily adapted by one of skill in the art.

These references to numerous published information and protocols regarding plant and animal chromosomal composition and structure, DNA manipulation, recombinant DNA expression, transfer of DNA into cells, including plants cells, and production of transgenic plants demonstrate the large volume

of information regarding tested and reliable procedures available at the time of filing of the instant application and thus evidence the advanced state of the art at the relevant time and the availability of such procedures for manipulation of plant cells, introduction of satellite artificial chromosomes into plant cells and production of transgenic plants from the satellite artificial chromosome-containing plant cells.

Presence of Working Examples

The specification provides numerous working examples and descriptions of the construction, isolation and transfer of satellite artificial chromosomes from various sources, such as plant systems, into various cells, such as a plant cell. Example 1, at page 69 of the specification, describes the culture of cell lines containing various artificial chromosomes and the transfection of cells with artificial chromosomes. Example 2, at page 75 of the specification, describes in great detail the preparation and maintenance of cell lines, including EC3/75, EC3/7C5 and EC3/7C6, which contain artificial chromosomes, as well as the assays used to monitor the expression of the *neo* gene encoded by the artificial chromosomes within the cells. Example 6, at page 92 of the specification, describes in great detail, methods for the generation of cell lines containing a megachromosome and detailed structural characteristics of this satellite artificial chromosome. Example 8, at page 113 of the specification, describes in great detail the *in vivo* replication of a megachromosome. Example 10, at page 124 of the specification, describes in great detail methods for the isolation of satellite artificial chromosomes from endogenous chromosomes based upon the atypical base content and/or size of the satellite artificial chromosome. Example 12, at page 140 of the specification, describes in great detail the preparation of vectors and plasmids, such as the λCF-7 and the λCF-7-DTA vectors and the pMCT-RUC and the pLNCX-ILRUC plasmids, for the targeted integration of heterologous DNA into artificial chromosomes. Example 13, at page 165 of the specification, describes methods for the microinjection of artificial chromosomes

into eukaryotic cells, and detection of expression of the encoded heterologous DNA (βGal) in cells injected with the DNA.

Predictability

As is known to those of skill in the art (described above), the level of knowledge and skill in the preparation, isolation, manipulation and transfer of heterologous DNA and artificial chromosomes as claimed in the instant application was high as of the effective filing date. Similarly, as discussed above, the level of knowledge and skill in the methods for production of transgenic plants from plant cells containing heterologous DNA also was high. Therefore, given the extensive teachings of the specification, in combination with what was known at the time the instant application was filed, it is not unpredictable that the satellite artificial chromosomes provided herein can be modified to produce non-mammalian satellite artificial chromosomes that can be introduced into plant cells, from which transgenic plants can be produced.

The pending claims are directed to methods for producing a transgenic plant that contains heterologous nucleic acid in which the heterologous nucleic acid is introduced into a plant cell by introducing a satellite artificial chromosome into the plant cell, and the plant cell is induced to generate a transgenic plant. The Office Action alleges that mammals and animals are not representative of plants in terms of chromosomes and chromatin structure. At page 4, paragraph 4 of the Office Action, the Office Action cites Ferl *et al.* (in Buchanan *et al.* "*Biochemistry & Molecular Biology of Plants*" (2000) American Society of Plant Physiologists, Rockville MD, pg. 324) and Lehninger ("*Biochemistry*" 2nd Edition (1976) Worth Publishers, New York, pg. 864) for the proposition that differences in base content of satellite DNA between plants and animals (AT-rich for animals and GC-rich for plants) result in differences in compactness of the satellite DNA. The Office Action then alleges that it is unclear how compact, densely H-bonded DNA affects satellite artificial chromosome activity and function.

The Office Action does not provide any suggestions based in the art that the composition of satellite DNA is linked to function. It is merely an observation that certain highly re-iterated sequences in plants and animals are either GC- or AT-rich, respectively, and hence have different physical properties as measured by physical biochemical methods to determine molecular density. Applicant respectfully submits that the physical properties of a class of DNA, as measured by buoyancy density centrifugation have little to do with its functionality as an element of a chromosome. Indeed, a chromosome is comprised of multiple components, including DNA and a variety of proteins, in a specific arrangement. For a chromosome to function, all of these components must be present in a functional form. Regardless, as stated in the specification, a satellite artificial chromosome is a chromosome that is substantially all heterochromatin (see, *e.g.*, the specification at page 19, lines 4-8). Heterochromatin, by definition, is a densely packed form of chromatin (see, *e.g.*, Voet & Voet in "*Biochemistry*" (1990), Wiley & Sons, New York, pg. 1033). Hence, absent evidence to the contrary, the mere fact that GC-rich DNA is more densely packed than AT-rich DNA should not effect the structure or function of a satellite artificial chromosome, which is already formed from a high percentage of densely packed heterochromatin. Rather, the highly condensed nature of heterochromatin, irrespective of whether it is of plant or mammalian origin, reinforces the similarities between plant and mammalian satellite artificial chromosomes that would enable generation of either species by following the methods and teachings of the specification.

Further, the Examiner is reminded that the structure of a chromosome contains additional features beyond simple DNA sequences. This includes the proteins that associate with the DNA to lead to the formation of the higher order chromatin structure of the chromosome. Plant and animals both contain histone and non-histone proteins, and it has been well-established that there is a large degree of conservation between animal and plant histone proteins. In particular,

it has now been established that animal and plant chromosomes contain proteins at the centromere region that are highly conserved (see, *e.g.*, Mole-Bajer *et al. Proc. Natl. Acad Sci. USA 87:* 3599-3603 (1990); and Houben *et al. Chromosome Res. 3:* 27-31 (1995)). These are particularly important proteins that bind to centromere regions and form the functional kinetochore. A functional kinetochore is essential for chromosome movement during mitosis. Numerous examples of similarity between kinetochore proteins of animal and plant species have been reported.

It is known that the composition of the kinetochore includes the centromere DNA sequences and proteins with kinetochore function. Antibodies to human kinetochore proteins cross react with plant kinetochore proteins as labeled on mitotic chromosomes (see, *e.g.*, Mole-Bajer *et al. Proc. Natl. Acad Sci. USA 87:* 3599-3603 (1990); and Houben *et al. Chromosome Res. 3:* 27-31 (1995)). Mole-Bajar *et al.* and Houben *et al.* demonstrated cross-reactivities of human antibodies from a patient with CREST against kinetochores of mitotic chromosomes of *Haemanthus katherinae* Bak and against the centromeric regions of mitotic chromosomes of Vicia faba (bean). In both plant species, putative homologs of the kinetochore protein SKP1 (suppressor of kinetochore protein 1p of yeast) were found. Accordingly, the art suggests that the essential elements of plant and animal kinetochores are highly similar and would be expected to behave in a similar fashion.

The Office Action also alleges that telomeres, origin of DNA replication and a centromere are required for the function of a satellite artificial chromosome, citing Willard (*Science 290:* 1308-1309 (2000)). The Office Action alleges that it is unclear what telomeres, origin of DNA replication and centromeres are necessary for non-animal satellite artificial chromosomes, whether additional components are required or how to isolate or construct functional satellite artificial chromosome in all cells, or non-mammalian cells (see, Office Action, page 5, paragraph 1).

Contrary to the assertion of the Office Action, the specification does provide methods for the development of a satellite artificial chromosome, which can be of animal or plant origin and comprise centromere-related sequences (see, *e.g.*, the specification at page 12, lines 5-22; and page 61, line 4, through page 64, line 18). In addition, methods for the production of repeated tandem arrays of DNA, such as telomeric DNA, are also provided in the specification (see, *e.g.*, page 14, line 4, through page 15, line 8; and page 64, line 19, through page 68, line 25). Utilizing the teachings of the specification and the teachings known in the art at the time of filing of the instant application, it is a simple matter to develop a satellite artificial chromosome in an animal cell, modified to contain sequences specific to the telomeric, origin of DNA replication or centromeric region of a different target cell and use said satellite artificial chromosome in the target cell type (*e.g.* plant cell).

Further, telomeric sequence, structure and function were well understood in the art at the time of filing (see, *e.g.*, Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); and Zakian *Science 270(5242):* 1601-1607 (1995)). Telomere sequences are highly conserved between animals and plants, comprising the same simple sequence CCCTAAA in humans and *Arabidopsis* (see, *e.g.*, Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); and Zakian *Science 270(5242):* 1601-1607 (1995)).

Furthermore, sequential and functional information regarding autonomously replicating sequences (ARS) in plants, which correspond to origins of DNA replication, was also known in the art at the time of filing (see, *e.g.*, Berlani *et al. Plant Mol. Biol. 11:* 161-162 (1988); Berlani *et al. Plant Mol. Biol. 11:* 173-182 (1988); and Eckdahl *et al. Plant Mol. Biol. 12:* 507-516

(1989)). In addition, the ability of an animal origin of replication to function in a plant cell was demonstrated at a functional level by Hadlaczky *et al.* (*In Vitro 16:* 647-650 (1980)) through the incorporation of label into animal DNA within a plant cell. The specification also provides a method for generating species specific satellite artificial chromosomes by adding a centromere from other species, including plants (see, *e.g.*, page 12, lines 5-22). Further, at the time of filing, exemplary sequence information for plant centromeres was available in the art (see, *e.g.*, Jiang *et al. Proc Natl Acad Sci USA 93:* 14210-14213 (1996); Kaszas *et al. EMBO J. 15(19):* 5246-5255 (1996); Frary *et al. Mol. Gen. Genet. 250:* 295-304 (1996); and Moore *et al. Chromosoma 105:* 321-323 (1997)). The methods outlined in these references provide a means to identify and isolate plant centromere DNA sequences. For example, Jiang *et al.* utilized fluorescence *in situ* hybridization to analyze the structure and molecular organization of the centromeric sequence in *Sorghum bicolor*.

The Office Action cites Ferl *et al.* in Buchanan *et al.* "Biochemistry and Molecular Biology of Plants" (2000) American Society of Plant Physiologists, Rockville MD, pg. 324; Willard *Science 290:* 1308-1309 (2000); Shen *et al. Current Biology 10:* 31-34 (2000); and Telenius *et al. Chromosome Res. 7*: 3-7 (1999), which allegedly demonstrate that centromeres of satellite artificial chromosomes show some species-specific behavior.

Furthermore, as discussed, such knowledge is not needed to practice the methods as claimed. As described above, cells, when treated as described in the instant application, generate the chromosomes from which the satellite artificial chromosomes are generated by virtue of amplification of regions of a chromosome. As discussed above, plant chromosomes have amplifiable regions (see, *e.g.*, U.S. Patent Nos. 6,355,860 and 6,100,092, attached hereto). Introduction of nucleic acids will result in amplification events. If the resulting cells are examined or sorted or treated and grown under selective conditions, cells containing satellite artificial chromosomes will be identified.

It is respectfully submitted that the references cited by the Examiner have no bearing on the predictability of the methods as instantly claimed, namely, methods for producing a plant cell such as a protoplast that contains heterologous DNA by introducing a satellite artificial chromosome into the cell and generating a transgenic plant therefrom. Willard provides the general state of the art with respect to components of artificial chromosomes and their assembly, but provides no teachings relevant to the production of satellite artificial chromosomes. Ferl *et al.* makes a general assertion that the sequences of the centromere among species varies. Ferl *et al.* does not, however, provide any teaching that this species variation impacts on the ability to produce satellite artificial chromosomes from any species, nor their introduction into cells of different species. In fact, Shen *et al.* and Telenius *et al.*, also cited by the Examiner in this regard, show that artificial chromosomes can be transferred between a wide variety of cells (*see, e.g.*, Shen *et al.* at page 33, column 2, lines 1-5; Telenius *et al.* at page 6, column 2, para. 3). Therefore, the species variation of centromere sequences, if any, has no bearing on the predictability of the methods as instantly claimed.

At page 5 of the Office Action, paragraph 2, the Office Action points to Avramova (*Plant Physiology 129:* 40-49 (2002)) as an assessment of the structure and function of heterochromatin in plants in comparison to its role in animals, which allegedly notes "heterochromatin is located at the nucleolar organizer and at the chromosome knob" in plants. Avramova continues, stating that (see, page 41, right column, second paragraph):

> at least three features make plant heterochromatin different from the animal chromatin: (a) absence of proteins similar to known heterochromatin proteins (see "Note added in Proof"); (b) location of potentially active genes in the knob structures and in the pericentromeric regions of plant genomes; and (c) different chromosomal environments for colinear genes in related species.

Avramova then states that "a family of approximately 20 putative DNA methyltransferases, containing a chromobox in the putative active center, is unique for plants" (see, page 44, left column, paragraph 2). The Office Action alleges that these passages show that patterns of heterochromatin differ between plants and animals. The Office Action further alleges that these differences coupled with differences in plant satellite DNA and plant centromeres and their animal counterparts demonstrate that "it is unpredictable that a plant satellite artificial chromosome, consisting of those components, would function as desired" in the claimed subject matter (page 7, last paragraph of the Office Action).

It is respectfully submitted that such selective reading of Avramova, in which statements regarding the comparison of structure and function of plant versus animal heterochromatin are taken out of context, has resulted in a mischaracterization of the reference that cannot validly be relied upon to support an allegation for unpredictability of plant satellite artificial chromosomes and their function as claimed in the instant application. For example, the Office Action points to a passage that states that heterochromatin in plants is located "at the nucleolar organizer and at the chromosome knobs" (page 5, paragraph 2). However, this passage begins: "In plants, **in addition to the centromeric and pericentromeric regions**, heterochromatin is located at the nucleolar organizer..." (page 40, left column). This statement, which was disregarded by the Office Action, indicates heterochromatin is also present in corresponding regions in plants and animals, specifically, the centromeric and pericentromeric regions.

Additionally, one of the differences between plant and animal heterochromatin that is cited in the Office Action, "the absence of proteins similar to known heterochromatin proteins (see "Note added in proof")" (page 41, right column) is actually a similarity (page 47, right column):

> Recent groundbreaking results in *Neurospora sp.* and *Arabidopsis* provided evidence for a connection between DNA methylation and

> histone H3 K9 methylation (H. Tamaru, E.U. Selker (2001) *Nature 414:* 277-283; J.P. Jackson, A.M. Lindroth, X. Cao, S.E. Jacobsen (2002) *Nature 416:* 556-560). In addition, the latter paper provided first evidence for the existence of histone H3 K9 methylation in plants, for the activity responsible for this modification, and for its connection to plant-specific CpNpG DNA methylation. A newly reported HP1-like factor from *Arabidopsis* (V. Gaudin, M. Libault, D. Poteau, T. Juul, G. Zhao, D. Lefebre, O. Grandjean (2001) *Development 128:* 4847-4858) is involved in mediating the control of CpNpG DNA methylation by H3 K9 methylation (Jackson *et al.*, 2002). Collectively, **these new results transform one of the differences between animals and plants, *i.e.*, absence of reported plant heterochromatin proteins, into a similarity** (emphasis added)

These statements, which were disregarded by the Office Action, indicate that plants do possess heterochromatin proteins with similar activities to those seen in animals.

Further, the Office Action points to plants having "a family of 20 methyltransferase enzymes unique to plants" (page 6, paragraph 1). Avramova, however, groups these enzymes into Class B of the chromodomain proteins, which **are not components** of heterochromatin (pages 42-44).

Additionally, according to the author, there are several similarities between plant and animal heterochromatin, as stated at page 41, right column:

> In summary, (a) in most species, the DNA moiety of heterochromatin is made of methylated repetitive DNAs of different types (including mobile elements) intermixed with low-copy and unique sequences; (b) a prerequisite for heterochromatin formation appears to be the structural organization of the repeats rather than the nature of the particular sequences, or their repetitive character; and (c) **based on the types and arrangement of the repetitive DNAs, heterochromatin in plants is similar to the heterochromatin in animals** (emphasis added)

The Office Action alleges that the "patterns" of heterochromatin differ between plants and animals, and correlates this difference and those described above to the unpredictability of the operability of a plant satellite artificial chromosome as claimed in the instant application. Avramova, however,

indicates that the pattern of the heterochromatin is not the delineating factor in its function. Rather, the structural organization of the repeats appears to be instrumental in the activity of the heterochromatin in plants and animals, which, according to Avramova, is similar between plants and animals.

At page 6, paragraph 2, of the Office Action, the Examiner cites Willard *Science 290:* 1308-1309 (2000); Brown *Trends in Biotech. 18:* 403 (2000); Perez *et al. Trends in Biotech 18*: 402-403 (2000); and Hadlaczky *Curr. Opin. Mol. Ther. 3:* 125-132 (2001)); for the proposition that "the transfer of large pieces of DNA between cells is a major problem in artificial chromosome technology."

As discussed above, Willard provides the general state of the art related to the assembly of **its** artificial chromosomes. Willard does not provide any teaching that the satellite artificial chromosomes, whose structure, generation, transfer into cells and stable expression are provided in the instant specification in great detail, would show any species-specific differences in the ability to be transferred between cells. Perez *et al.* and Hadlaczky *et al.* comment on the general inefficiency of transfer of artificial chromosomes, but then go on to say that satellite artificial chromosomes appear to overcome these problems and are especially well suited for production in different cell types. Thus, the references cited by the Examiner in fact describe how the problem of transfer of large pieces of DNA between cells can be overcome. Brown merely responds cynically to the assertions in Perez *et al.* that satellite artificial chromosomes are transferred efficiently between cells, but provides no evidence to refute the methods of Perez *et al.* As discussed above, other references cited by the Examiner (*see, e.g.*, Shen *et al.* and Telenius *et al.*) also demonstrate efficient transfer of large pieces of DNA between cells. Furthermore, although not needed, the accompanying DECLARATION under 37 C.F.R. § 1.132 of Steven F. Fabijanski demonstrates that by following the steps of the instant methods, one of skill in the art can introduce satellite artificial chromosomes into plant

protoplasts using well known methods, including microcell-mediated fusion and lipid-mediated transfection.

Therefore, it is respectfully submitted that a high degree of conservation between or knowledge about the essential functional elements of plant and animal chromosomes, such as the telomeres, the kinetochore and the autonomously replicating sequences (ARSs) was known at the time of filing of the instant application, to render the instantly claimed methods predictable. Further, should a modification be required, such as alteration of the centromeric sequence, methods for modifying the mammalian satellite artificial chromosomes are disclosed in the instant application, including identifying and adding centromere DNA sequences from various organisms, including plants, and known to those of skill in the art. Thus, Applicant respectfully submits that one of skill in the art could, using the teachings of the specification and the information available in the art, identify and incorporate telomeric, centromeric and autonomously replicating sequences for use in a satellite artificial chromosome operable in a plant cell. Furthermore, methods to modify the satellite artificial chromosome described in the instant application were adequately described in the specification (see, *e.g.*, page 39, line 26, through page 40, line 21; and pages 150-157), where homologous recombination is used to insert new DNA sequences into a satellite artificial chromosome. Accordingly, methods to generate and modify a satellite artificial chromosome to contain plant telomeres, centromere and autonomously replication sequences has been described.

The Office Action also alleges that the specification of the instant application does not provide guidance for the manipulation of the enabled mammalian satellite artificial chromosomes to prepare a satellite artificial chromosome operable in cellular systems other than animal cells.

To the contrary, the specification describes in great detail, methods for the addition of heterologous DNA sequences, such as DNA derived from plants,

to an animal satellite artificial chromosome. Furthermore, the methods by which the animal satellite artificial chromosomes are generated are applicable to any eukaryotic species; these methods are not species specific.

Applicant respectfully submits that it is not a requirement *a priori* to make changes to a mammalian satellite artificial chromosome to function as claimed within a plant cell. The transfer and function of an mammalian-derived satellite artificial chromosome in a plant system is within the scope of the methods as described in the instant application and the knowledge of those of skill in the art. For example, methods for transferring naturally occurring chromosomes between animal and plant cells are known in the art (see, *e.g.*, Szabados *et al. Planta 151:* 141-145 (1981)). Szabados *et al.* demonstrated that isolated mitotic animal chromosomes can be transferred into plant cells by simple techniques, such as by producing a fusion product between a plant protoplast and isolated chromosomes from animal cells. Thus, the art clearly demonstrates that transfer of animal chromosomes into plant cells can be achieved. Further, numerous methods for the transfer of heterologous DNA, including artificial chromosomes, into non-mammalian cells, such as insect, avian and plant cells, are referenced throughout the instant application (see, *e.g.*, page 10, line 30, through page 11, line 10; page 48, line 11, through page 51, line 26; page 52, line 11, through page 55, line 3; page 70, line 14, through page 72, line 27; Example 11, beginning on page 137; Example 13 beginning on page 165; and Example 14, beginning on page 176).

The art further demonstrates that once transferred into a plant cell environment, the animal chromosome is still capable of undergoing DNA synthesis (see, *e.g.*, Hadlaczky *et al. In Vitro 16:* 647-650 (1980)). By monitoring the incorporation of ^{3}H thymidine, Hadlaczky *et al.* demonstrated that *Drosophila* nuclei were able to both synthesize DNA and divide in the mixed cytoplasm. Accordingly, at the time of filing of the instant application, the art indicates that animal chromosomes can be transferred into plant cells and that

these chromosomes are operable for DNA replication and mitotic division within the plant cell. Therefore, there is precedence in the art that a satellite artificial chromosome that is derived from an animal cell can be transferred into a plant cell and be operable as claimed in the instant application (*e.g.*, in a transgenic plant). The Office Action also alleges that the Applicant teaches an animal satellite artificial chromosome in a mammalian cell but does not provide the guidance to make necessary changes to a mammalian satellite artificial chromosome to derive a non-mammalian satellite artificial chromosome thus requiring undue experimentation.

In particular, using a satellite artificial chromosome for various applications such as construction of libraries and delivery of new genetic sequences to cells are fully enabled within the specification (see, *e.g.*, the specification, at page 61, lines 5-25). This passage describes the use of a satellite artificial chromosome, to identify functional centromeres from other organisms. In addition, the specification also teaches methods for isolation (see, *e.g.*, page 41, line 5, through page 42, line 3; page 128, line 21, through page 135, line 21) and delivery of satellite artificial chromosomes to various cells, such as, for example, via microcell fusion in mammals (see, *e.g.*, page 70, line 15, through page 72, line 27), insect cells (see, *e.g.*, page 139, line 5, through page 140, line 10) and avian cells (see, *e.g.*, page 178, line 17, through page 179, line 30). Thus, Applicant submits that it is routine procedure for one of skill in the art to adapt the methods disclosed in the instant application to effect the delivery of a satellite artificial chromosome into a plant cell for the production of a transgenic plant.

Conclusion

In light of the extensive teachings and examples in the specification, the high level of skill of those in this art, the knowledge of those of skill in the art, the fact that it is predictable that satellite artificial chromosomes from various sources can be produced and introduced into a selected cell (*e.g.*, a plant cell)

and the breadth of the claims, it would not require undue experimentation for one of skill in the art to practice the methods as claimed.

Accordingly, a consideration of the factors enumerated in Ex parte Forman leads to the conclusion that undue experimentation would not be required to introduce a satellite artificial chromosome into a plant cell and grow the cell under conditions to produce a transgenic plant, based on the disclosure in the specification.

Declaration

Notwithstanding the above arguments, to evidence that the methods as claimed operate as claimed, attached is a DECLARATION under 37 C.F.R. §1.132 of Steven F. Fabijanski. The DECLARATION shows that when using the teachings of the application, animal satellite artificial chromosomes can be delivered to plant cells as claimed.

Also, although this DECLARATION is submitted to rebut the Examiner's assertions of inoperativeness, it also further evidences enablement. It is noted that the level of skill in the biotechnical arts is recognized to be high (see, *e.g.*, *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). Further, methods for performing the various steps of the claimed methods, such as preparation of microcells, microcell-mediated fusion, lipid-mediated transfection and techniques for the detection of specific DNA sequences (*e.g.*, satellite artificial chromosomes) in recipient cells were known to the skilled artisan at the time of filing.

In conducting and directing the experiments in the DECLARATION, Dr. Steven Fabijanski followed the teachings in the application and standard methods as were those of skill in the art as of the earliest priority date of the above-captioned application. Since those of skill in this art typically have advanced degrees, Dr. Fabijanski, who has a Ph.D. degree, is representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol.

The DECLARATION demonstrates that using the procedures as taught in the above-captioned application, animal satellite artificial chromosomes can be delivered to plant cells. The results demonstrate that delivery of animal satellite artificial chromosomes to plant cells can be achieved by methods well known to those of skill in the art, including microcell-mediated fusion and lipid-mediated transfection.

Specifically, the DECLARATION of Fabijanski demonstrates that (i) mammalian satellite artificial chromosomes can be transferred to plant protoplasts by microcell-mediated fusion; (ii) isolated mammalian satellite artificial chromosomes can be transferred to plant protoplasts by lipid-mediated transfer; and (iii) satellite artificial chromosome material can be detected in plant cells by Southern hybridization, PCR or FISH analysis for at least 16 weeks following satellite artificial chromosome transfer.

The DECLARATION describes the transfer of an animal satellite artificial chromosome into tobacco protoplasts and *Arabidopsis* protoplasts using microcell-mediated transfer with microcells prepared from a satellite artificial chromosome-containing murine cell line. The DECLARATION also describes transfer of isolated animal satellite artificial chromosomes into rice plant protoplasts using lipid-mediated transfection. The DECLARATION demonstrates that transferred satellite artificial chromosome material can be detected in plant cells using a variety of methods, including DAPI staining, Southern hybridization using artificial chromosome probes such as mouse major satellite DNA and PCR analysis using satellite sequences known to exist on the satellite artificial chromosome. The DECLARATION also demonstrates that satellite artificial chromosomes can be detected using FISH analysis of plant protoplast nuclei isolated after microcell fusion or transfection methods.

The results of these experiments demonstrate that the methods provided in the above-referenced application can be used to deliver satellite artificial chromosomes into plant cells where the satellite artificial chromosome can be an

animal satellite artificial chromosome. Therefore, the DECLARATION provides further evidence that the disclosure in the above-captioned application enables methods for introducing animal satellite artificial chromosomes into plant cells. The DECLARATION demonstrates element-for-element and step-for-step that, by following the teachings in the application, one can (i) transfer satellite artificial chromosomes into plant cells where the satellite artificial chromosome can be an animal satellite artificial chromosome; and (iii) detect satellite artificial chromosome material in plant cells for at least 16 weeks following satellite artificial chromosome transfer. Accordingly, Applicant respectfully submits that the claims are commensurate in scope with the Applicant's discovery and its disclosure within the above-captioned application.

Policy Considerations

A significant portion of the grounds for the rejection of the claims under 35 U.S.C. § 112, first paragraph, is based on the alleged unpredictability of the art of the production of satellite artificial chromosomes from any source, particularly a plant cell, and their introduction into any plant cell for the production of a transgenic plant. The Office Action alleges that the specification does not teach the production of a "universal" satellite artificial chromosome that would be operable in any cell type, including a plant cell. The claims are directed to the method of producing a transgenic plant that includes introducing a satellite artificial chromosome into the plant protoplast, and growing the plant protoplast cell under conditions to produce a transgenic plant. The specification fully enables one of skill in the art to accomplish this end result. In other words, the specification teaches one of skill in the art to "make and use" a satellite artificial chromosome for the introduction of heterologous nucleic acid into a cell. The specification provides agents, such as cell lines and vectors, and methods for the production of satellite artificial chromosomes and the transfer of the satellite artificial chromosome into a cell, and describes their use *e.g.*, in gene therapy and the production of transgenic animals and plants

that possess desired traits, such as resistance to disease. In fact, the application does teach a "universal" method for production of satellite artificial chromosomes and methods for transfer of such chromosomes into any cell type.

Accordingly, the issue of whether the specific instant claims are enabled by the specification should not turn on the state of the art regarding the similarities between plant and animal chromosomal composition and function, as generally discussed on pages 4-7 of the Office Action. Instead, the relevant question with regard to enablement of the subject matter of the instant claims is whether the particular steps and materials of the claimed methods are described in the specification in such a way as to enable one skilled in the art to make and use the subject matter **as claimed**. Therefore, as discussed above, the instantly claimed methods are described in detail in the application to the satisfaction of 35 U.S.C. § 112, first paragraph.

Further, as taught in the above-captioned application, any methods known in the art pertaining to introduction of foreign genes carried in traditional, standard sources (such as genes harbored in expression vectors) into cells for any variety of purposes, *e.g.*, gene therapy, protein production and the generation of transgenic animals and plants, can be applied in similar fashion to the introduction of satellite artificial chromosomes, such as plant satellite artificial chromosomes into plant cells. The application describes and demonstrates that once the artificial chromosomes are generated and isolated and/or introduced into cells, then any known procedure that has previously been carried out with any heterologous gene from any source is applicable to utilization of artificial chromosomes carrying foreign genes of interest. The application is replete with descriptions of numerous uses of satellite artificial chromosomes and minichromosomes. The descriptions of the many ways in which the artificial chromosomes can be used include references to reported procedures for introducing exogenous nucleic acids into cells.

Applicant is entitled to claims that are commensurate in scope not only with what Applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the Applicant has disclosed. In the above-captioned application, Applicant provides a pioneering discovery and discloses to the public methods and compositions for the controlled introduction and stable extra-genomic maintenance of large heterologous DNA fragments in cells without disruption of the inherent genome and, likewise, without the otherwise uncontrollable influences that the genomic DNA can have on the expression of the heterologous DNA. The artificial chromosomes disclosed in the application can be produced and used to express heterologous genes in cells, as is taught and specifically exemplified in the specification. It is clear that Applicant's discovery is of a pioneering nature, and, as such, is entitled to broad claim protection.

As a broad body of knowledge is available in the area of molecular biology and preparation of nucleic acid for use in the manipulation of chromosomal components, including many technical procedures covering the manipulation of DNA and recombinant DNA techniques, it would be unfair, unduly limiting and contrary to the public policy upon which the patent laws are based to require Applicant to limit these claims to a particular satellite artificial chromosome or cell type. See, e.g., In re Goffe, 542 F.2d 801, 166 USPQ 85 (CCPA 1970):

> for the Board to limit appellant to claims involving the specific materials disclosed in the examples so that a competitor seeking to avoid infringing the claims can merely follow the disclosure and

and make routine substitutions "is contrary to the purpose for which the patent system exists - to promote progress in the useful arts".

> The public purpose on which the patent law rests requires the granting of claims commensurate in scope with the invention disclosed. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions"

In re Sus and Schafer, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301, at 304.

To require Applicant to further limit the claims would permit those of skill in the art to practice what is disclosed in the specification but avoid infringing claims so-limited. If Applicant is required to limit the claims to only the aforementioned satellite artificial chromosomes and cell types, then those of skill in the art could by virtue of the teachings of this application readily practice what is claimed by producing the satellite artificial chromosomes from a different source and/or introduce it into a varied cell type and practice what is disclosed in the application, but avoid infringing such limited claims. To permit that is simply not fair. The instant application exemplifies the preparation of satellite artificial chromosomes from mammals, such as mouse chromosomes 1 and 7, and their transfer into mammalian cells, such as mouse fibroblast cells, chinese hamster ovary cells and human lymphocyte cells. Further, the instant application in light of the knowledge of those of skill in the art provides adequate guidance for the preparation of plant satellite artificial chromosomes and for the transfer of satellite artificial chromosomes of any species into plant or animal cells. Having done so, it is now routine to for others to prepare artificial chromosomes from other sources, such as plants, and prepare alternative host cells containing the satellite artificial chromosome. Those of skill in the art should not be permitted to make such minor modifications by substitution of a different source or host and avoid infringing such claims.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: HADLACZKY *et al.*
Serial No.: 09/724,726
Filed: November 28, 2000
Customer No.: 24961
Confirmation No: 7776
For: *ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS FOR PREPARING ARTIFICIAL CHROMOSOMES*
Art Unit: 1638
Examiner: Helmer, G.L.

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07/16/2003
Date

Jonathan Ong

ATTACHMENTS TO RESPONSE TO OFFICE ACTION

1. Marked-up claims (37 C.F.R. § 1.121)
2. DECLARATION of FABIJANSKI pursuant to 37 C.F.R. §1.132.

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07/16/2003
Date

Jonathan Ong

MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claim 50 as follows:

50. (Twice Amended) A method for producing a transgenic plant, comprising introducing a satellite artificial chromosome (SATAC) into a plant protoplast; and

growing the protoplast [cell] under conditions to produce a transgenic plant.